

AD-A231 115

DTIC FILE COPY

(2)

OFFICE OF NAVAL RESEARCH

Contract N00014-88-K-0401

Technical Report No. 008

**Electron Transfer between Glucose Oxidase and Electrodes via
Redox Mediators Bound with Flexible Chains to the Enzyme Surface**

**Wolfgang Schuhmann, Hanns-Ludwig Schmidt
Lehrstuhl für Allgemeine Chemie und Biochemie
Technische Universität München
D-8050 Freising-Weihenstephan, FRG**

and

Timothy J. Ohara, and Adam Heller*
Department of Chemical Engineering
The University of Texas at Austin
Austin, Texas 78712-1062

**Accepted for Publication in
Journal of the American Chemical Society
February 1991**

January 11, 1991

**DTIC
ELECTED
JAN 22 1991
S B D**

**Reproduction in whole, or in part, is permitted for any purpose of the
United States Government.**

**This document has been approved for public release and sale; its
distribution is unlimited.**

SECURITY CLASSIFICATION OF THIS PAGE

Form Approved
OMB No. 0704-0188

REPORT DOCUMENTATION PAGE							
1a. REPORT SECURITY CLASSIFICATION Unclassified		1b. RESTRICTIVE MARKINGS					
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release and sale; its distribution is unlimited.					
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE							
4. PERFORMING ORGANIZATION REPORT NUMBER(S) TECHNICAL REPORT NO. 008		5. MONITORING ORGANIZATION REPORT NUMBER(S)					
6a. NAME OF PERFORMING ORGANIZATION Dept. of Chemical Engineering University of Texas at Austin	6b. OFFICE SYMBOL (if applicable)	7a. NAME OF MONITORING ORGANIZATION Department of Sponsored Projects University of Texas at Austin					
6c. ADDRESS (City, State, and ZIP Code) Austin, TX 78712-1062	7b. ADDRESS (City, State, and ZIP Code) P.O. Box 7726 Austin, TX 78713-7726						
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research	8b. OFFICE SYMBOL (if applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER					
8c. ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217	10. SOURCE OF FUNDING NUMBERS <table border="1"> <tr> <td>PROGRAM ELEMENT NO.</td> <td>PROJECT NO.</td> <td>TASK NO.</td> <td>WORK UNIT ACCESSION NO.</td> </tr> </table>			PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.				
11. TITLE (Include Security Classification) Electron Transfer Between Glucose Oxidase and Electrodes via Redox Mediators Bound with Flexible Chains to the Enzyme Surface							
12. PERSONAL AUTHOR(S) Wolfgang Schuhmann, Timothy J. Ohara, Hanns-Ludwig Schmidt, Adam Heller							
13a. TYPE OF REPORT Technical	13b. TIME COVERED FROM <u>7/1/89</u> TO <u>1/1/91</u>	14. DATE OF REPORT (Year, Month, Day) 1991 1 11	15. PAGE COUNT				
16. SUPPLEMENTARY NOTATION							
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)					
FIELD	GROUP	SUB-GROUP					
19. ABSTRACT (Continue on reverse if necessary and identify by block number) <p>Electrical communication between redox centers of glucose oxidase and vitreous carbon electrodes is established through binding to oligosaccharides, at the periphery of the enzyme, ferrocene functions pendant on flexible chains. Communication is effective when the chains are long (> 10 bonds), but not when the chains are short (< 5 bonds). When attached to long flexible chains the peripherally bound relays penetrate the enzyme to a sufficient depth to reduce the electron transfer distances between a redox center of the enzyme and the relay and between the relay and the electrode, thereby increasing the rate of electron transfer. (25) * Electrodes, * Glucose, * Oxidoreductases.</p>							
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input type="checkbox"/> UNCLASSIFIED/UNLIMITED <input checked="" type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION					
22a. NAME OF RESPONSIBLE INDIVIDUAL Adam Heller		22b. TELEPHONE (Include Area Code) (512) 471-8874	22c. OFFICE SYMBOL				

TXT06
PAR12

SEN13 9 available grade and used without further purification. Unless otherwise noted, all experiments were performed at room temperature in a standard aqueous buffer solution containing 100 mM phosphate and 200 mM NaCl at pH 7.2.

PAR15

SEN03 1 **Electrodes and Equipment.** Electrochemical measurements were performed with an EG&G Princeton Applied Research 175 universal programmer, a Model 173 potentiostat, and a Model 179 digital coulometer.
SEN04 4 SEN06 5
SEN09 1 SEN12 3
SEN11 2 SEN15 38
SEN18 15
SEN19 6
SEN20 7
SEN21 8

The signal was recorded on Kipp and Zonen Y-Y-Y recorder. Glassy carbon rods (Sigradur, 3-mm diameter) sealed with epoxy resin into glass were polished prior to use on a polishing cloth sequentially with alumina of decreasing particle size (1, 0.3, 0.5 μm), sonicated, rinsed with distilled water, and then dried in air. A single-compartment electrochemical cell was used with an aqueous KCl/saturated calomel (SCE) reference electrode and a platinum counter electrode. All potentials are referred to this reference electrode (+244 mV vs NHE).

PAR18

SEN03 1 **Synthesis of Ferrocene Derivatives.** The ferrocene derivatives with different spacer lengths were synthesized as shown in Figure 1. A 4-fold excess of the appropriate diamine was heated in 100 mL of DMF to 100 °C, and 500 mg of ferrocenecarboxaldehyde dissolved in 50 mL of DMF was added dropwise within 1 h to prevent formation of the bridged diferrocene compound. After another hour an excess of sodium borohydride in water was dropped into the solution, and the reaction mixture was stirred for an additional hour at room temperature. The solvent mixture was rotavaporated to dryness and the residue extracted with dichloromethane and separated through a silica column (1.5 cm \times 30 cm). A first fraction—the bridged ferrocene—was eluted with dichloromethane, the main fraction with dichloromethane/methanol 10:1.
SEN12 42 SEN15 21
SEN18 25 SEN21 1
SEN24 21

The solvent was evaporated to dryness, the residue dissolved in diethyl ether, and the hydrochloride precipitated by bubbling gaseous hydrochloric acid through the solution. All compounds show the expected ^1H NMR spectra.

PAR21

SEN03 1 **Preparation of Ferrocene-Modified Glucose Oxidase.** The oxidation of the enzyme-bound sugar residues was performed with sodium periodate according to established procedures.¹² The ferrocenes were attached to the aldehyde groups formed thus on the outer enzyme surface via Schiff bases, which were reduced with sodium borohydride subsequently (Figure 2). The modified enzyme was isolated from low molecular weight compounds and desalts by gel chromatography (Sephadex G25 equilibrated with water, column 2.5 cm \times 20 cm). The volume was reduced by means of ultrafiltration through a membrane (Amicon PM30, MWCO 30000), and the modified enzyme was freeze-dried. To verify that the unreacted ferrocenes were not electrostatically bound to the enzyme, the freeze-dried product was redissolved and extracted with copious amounts of a solution containing 0.1 M phosphate and 0.1 M NaCl at pH 7.1 in an ultrafiltration cell. After refreeze-drying, the electrochemical characteristics of the modified enzyme were unchanged, confirming the absence of noncovalently bound ferrocenes. Determination of the amount of aldehyde groups at the enzyme surface was performed by a procedure of Sawicki et al.¹³ The activity of the lyophilized enzymes was determined spectrophotometrically by the α -dianisidine/peroxidase assay.¹⁴ The labeling of the enzyme with ferrocenes was evaluated by atomic absorption spectroscopy and by coulometry.

TXT09

SEN03 1 **Results and Discussion**

PAR24

SEN03 1 **Synthesis of Ferrocene-Labeled Glucose Oxidase.** Glucose oxidase (EC 1.1.3.4 from *Aspergillus niger*) is a dimer glycoprotein with a molecular mass of 186 000 daltons. The oligosaccharide chains, which form a hydrophilic periphery, represent ~12% of its weight. Oxidation of these with periodate¹² has been used to provide peripheral aldehyde groups for the immobilization of glycoenzymes to polymeric supports¹⁵ or to electrode surfaces.¹⁶
SEN06 3 SEN09 13
SEN12 13
SEN15 1
SEN18 22
SEN21 19
SEN24 11
SEN27 16
SEN30 24
SEN33 34
SEN36 29

Analogously, we have now applied this method to bind ferrocene derivatives with different spacer lengths to the surface of glucose oxidase. The periodate oxidation of glucose oxidase was investigated with respect to the number of aldehyde functions obtained and the decrease of enzymatic activity during the reaction. As expected, the aldehyde concentration increased when the reaction times were longer and the enzymatic activity decreased. Optimal results were obtained at a reaction time of 1 h and a periodate concentration of >20 mM, the conditions of our experiments. The number of aldehyde groups, introduced upon oxidation with 20 mM sodium periodate, was determined spectrophotometrically after its reaction with 3-methyl-2-benzothiazolinone hydrazone hydrochloride, following a procedure of Sawicki et al.¹³ Assuming that the extinction coefficient reported for the hydrazones of aldehydes formed from mannitol ($\epsilon = 95\,000 \text{ L mol}^{-1} \text{ cm}^{-1}$) is similar to that of the hydrazones of the oxidized enzyme, we estimate 6.4 aldehyde groups per enzyme molecule.¹⁷ However, because polysaccharides do not react as completely as monosaccharides with this hydrazone, and because the extinction coefficient for the aldehydes derived from mannitol is higher than that of other sugars, this estimate may be low. The functionalized

FIG 1 (006,15-16)

FNT 12

FIG 2 (009,28-29)

FNT 13

FNT 14

FNT 15, FNT 16

FNT 17

TXT09
PAR24

4 enzyme used for the covalent binding of the different ferrocene
14 compounds showed an activity of 66 units mg⁻¹.

PAR27

SEN03 1 As the rate of electron transfer decays exponentially with the
12 distance of the involved redox centers, a significant influence of
22 the spacer length between enzyme surface and mediator on the
32 electron-transfer properties of the modified enzymes in question
was expected.

SEN06 40 To evaluate the effect of chain length on the
11 effectiveness of electron transfer to electrodes, we prepared the
20 series of ferrocene-derivatized enzymes shown in Table I (com-
pounds 1-7).

SEN09 28 The amino-functionalized ferrocene derivatives have
7 been synthesized through the reaction sequence shown in Figure
16 1 and purified by column chromatography. Following IO₄⁻ ox-
4 idation of the oligosaccharide residues on the enzyme, the resulting
14 aldehyde groups were reacted with ferrocene amines, to form
23 Schiff bases. These were reduced with NaBH₄ to the secondary
SEN12 20 amines (Figure 2). Binding of amino spacer modified ferrocene
17 derivatives to the surface of the functionalized glucose oxidase
1 amines (Figure 2). Binding of amino spacer modified ferrocene
17 did not lead to a further decrease of enzymatic activity (see Table
1 I).

PAR30

SEN03 1 **Electrochemical Investigations of Ferrocene-Modified Glucose**
SEN06 7 **Oxidase.** The results of the electrochemical measurements are

SEN09 9 summarized in Figure 3 and Table I. The cyclic voltammograms
5 shown in Figure 3 were run at 2 mg mL⁻¹ concentration of the
18 ferrocene-modified enzymes 1-7 in 0.1 M phosphate buffer (pH
27 7.2).

SEN12 27 The three-electrode cells were equipped with a glassy carbon
11 (3-mm diameter) working electrode, a platinum wire counter
19 electrode, and a KCl-saturated calomel reference electrode.

SEN15 1 Catalase was added to the solutions (200 units mL⁻¹) to decompose
13 any hydrogen peroxide that might be formed in the presence of
SEN18 24 residual oxygen. Curve 1 of Figure 3 shows the cyclic voltam-
10 mograms of a solution of compound 1 in buffer (a) without glucose
SEN21 22 and (b) with 40 mM glucose. Curves 2 and 3 show the cyclic
9 voltammograms observed under identical conditions for compounds
SEN24 16 2 and 4, respectively. The limiting currents, normalized for the
8 amount of attached ferrocene, increase with chain length (Table
SEN27 17 I). Notable enhancement of the catalytic current is observed in
11 compound 7, where $i = 6.5 \mu\text{A}$, i.e., the current density reaches
90 $\mu\text{A cm}^{-2}$.

PAR33

SEN03 1 **Electron-Transfer Model.** A peripherally attached redox me-

SEN06 6 diator may accept electrons through either an intramolecular or
15 an intermolecular process (Figure 4), acting in the latter as a
26 conventional diffusing mediator. For example, mediation by

SEN12 6 ferrocene-modified albumin has been reported.⁶ The dominance
4 of the intramolecular electron-transfer process in the case of
13 enzymes with long chains was established through the following

SEN15 22 experiment. Enzymes 1 and 4 were partially deactivated by 6 M
12 urea (4 h, 25°), and then separated from the urea by gel-per-
SEN18 21 mation chromatography. Their catalytic currents (i' (Table II))

SEN21 19 were measured at an enzyme concentration of 1 mg mL⁻¹ under
6 conditions identical with those for i_{cat} in Table I. Then 1 mg mL⁻¹

SEN24 16 native glucose oxidase was added, and the catalytic current (i''_{cat}
Table II) was determined. If the process were entirely inter-
7 molecular, i''_{cat} would have been equal to or greater than i_{cat}

18 because the concentration of the electron-transfer mediator is
26 unchanged and both the concentration and relative catalytic ac-
34 tivity of the enzyme are increased (note in Table I that 1 and 4
48 retain, respectively, 0.27 and 0.45 of the native enzyme's activity).

SEN27 1 If the process were entirely intramolecular, addition of native
11 enzyme would not have changed the catalytic current seen with
SEN30 21 the deactivated enzyme (i'_{cat} , Table II). Measurement of the
5 catalytic current in the presence of deactivated 1 and 4 with native
17 enzyme added shows that in the case of 1, where the chain is short,
31 the current approaches i_{cat} for the enzyme prior to deactivation,
41 i.e., that the process of electron transfer either has a substantial
SEN33 32 intermolecular component or is entirely intramolecular. For
3 compound 4, made with long chains, i'_{cat} , the current observed
11 with the partially deactivated enzyme plus native enzyme (470
22 nA), remains much below the 2800-nA catalytic current of the
32 enzyme prior to its partial deactivation and is only marginally
42 higher than the 350-nA current of the partially deactivated enzyme
4 (Table II). This indicates that when the spacer chain is long the
SEN36 52 process is dominantly intramolecular. We thus conclude that the
7 increase in catalytic currents with increase in chain length (Table
17 I and Figure 3) originates in enhanced intramolecular electron
26 transfer from the enzyme's redox centers to the chain-attached
SEN42 35 mediator and, via the mediator, to the electrode. Our observations
4 do not allow us to define the extent of electron transfer by a
17 dynamic process, where the chain-pendant mediator swings "in"

relay

TBL I (006,27-28)

FIG 3 (006,12-13)

FIG 4 (006,19-20)

TBL II (018, 7-8)

Accession Per	
NTIS GRAAI	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By _____	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

relay



TXT09
PAR33

23 and "out" of the enzyme, and a static process, where the relay
 37 is reasonably stationary, i.e., is bound by hydrophobic or elec-
 46 trostatic interaction to a specific region in the protein.

TXI12
PAT36

SEN00 1 **Acknowledgment.** We thank Dr. B. A. Gregg for the prepa-
SEN06 10 ration of (aminoethyl)ferrocene and many helpful discussions. The
 3 work at the University of Texas at Austin is supported by the
 15 Office of Naval Research, the Welch Foundation, and the Texas
 20 Advanced Research Program. The work at the Technical
 7 University of Munich is supported by the Bundesministerium für
 16 Forschung und Technologie (BMFT), Projektträger Biotechno-
 21 logie, FRG. This collaborative study was performed at the
 9 University of Texas.

SYF03
SEN00 1 ¹Technische Universität München.
SYF06
SEN00 1 ¹The University of Texas.

FNN02
FNP03
SEN03
FNN03
FNP06
SEN03
FNN04
FNP09
SEN03
FNN05
FNP12
SEN03
FNN06
FNP15
SEN03
FNN07
FNP18
SEN03
FNN08
FNP21
SEN03
FNN09
FNP24
SEN03
SEN09
SEN12
FNN10
FNP27
SEN03
SEN09
SEN12
FNN11
FNP30
SEN03
SEN06
SEN09
FNN12
FNP33
SEN03
FNN13
FNP30
SEN03
SEN06
FNN14
FNP39
SEN03
FNN15
FNP42
SEN03
FNN16
FNP48
SEN03
FNN17
FNP48
SEN03
SEN06
FNN18
FNP51
SEN03

1 (1) Heller, A. *Acc. Chem. Res.* 1990, **23**, 128.

1 (2) Marcus, R. M.; Satin, N. *Biochim. Biophys. Acta* 1985, **81**, 265.

1 (3) Clark, L. D., Jr.; Lyons, C. *Ann. N.Y. Acad. Sci.* 1962, **102**, 29.

1 (4) (a) Aleksandrovskii, Y. A.; Bezhikina, L. V.; Rodionov, Y. U. *Biokhimiya* 1981, **708**. (b) Kulya, J. J.; Cenac, N. K. *Biochim. Biophys. Acta* 1983, **744**, 57. (c) Senda, M.; Ikeda, T.; Hiasa, H.; Miki, K. *Anal. Sci.* 1986, **2**, 501. (d) Cass, A. E. G.; Davis, G.; Green, M. J.; Hill, H. A. O. J. *Electroanal. Chem.* 1985, **190**, 117. (e) Cass, A. G.; Davis, G.; Francis, G. D.; Hill, H. A.; Aston, W. J.; Higgins, I. J.; Plotkin, E. V.; Scott, L. D. L.; Turner, A. P. F. *Anal. Chem.* 1984, **56**, 667. (f) Kulya, J. J. *Biosensors*, 1986, **2**, 3. (g) Albery, W. J.; Bartlett, P. N.; Cass, A. E. G. *Philos. Trans. R. Soc. London B* 1987, **316**, 107.

1 (5) Schuhmann, W.; Wohlschlager, H.; Lammett, R.; Schmidt, H.-L.; Löffler, U.; Wiemhofer, H.-D.; Göpel, W. *Sensors Actuators B* 1990, **1**, 371.

1 (6) Mizutani, F.; Asai, M. *Denki Kagaku* 1988, **56**, 1100.

1 (7) Schuhmann, W., unpublished results.

1 (8) (a) Degani, Y.; Heller, A. *J. Am. Chem. Soc.* 1989, **111**, 2357. (b) Gregg, B. A.; Heller, A. *Anal. Chem.* 1990, **62**, 258. (c) Pishko, M. V.; Kataki, I.; Lindquist, S.-E.; Ye, L.; Gregg, B. A.; Heller, A. *Angew. Chem., Int. Ed. Engl.* 1990, **39**, 82. (d) Hale, P. D.; Inagaki, T.; Karan, H. I.; Okamoto, Y.; Skotheim, T. A. *J. Am. Chem. Soc.* 1989, **111**, 3482.

1 (9) (a) Degani, Y.; Heller, A. *J. Phys. Chem.* 1987, **91**, 1285. (b) Degani, Y.; Heller, A. *J. Am. Chem. Soc.* 1988, **110**, 2615. (c) Heller, A.; Degani, Y. in *Redox Chemistry and Interfacial Behavior of Biological Molecules*; Dryhurst, G., Niki, K., Eds.; Plenum Press: New York, 1988; p 151. (d) Bartlett, P. N.; Whitaker, R. G.; Green, M. J.; Frew, J. *J. Chem. Soc., Chem. Commun.* 1987, 1603.

1 (10) (a) Mosbach, K.; Guilford, H.; Ohlson, R.; Scott, M. *Biochem. J.* 1972, **127**, 627. (b) Schmidt, H.-L.; Grenner, G. *Eur. J. Biochem.* 1976, **67**, 295. (c) Grenner, G.; Schmidt, H.-L.; Voelkl, W. *Hoppe-Seyler's Z. Physiol. Chem.* 1976, **357**, 887.

1 (11) Lednicer, D.; Lindsey, J. K.; Hauser, C. R. *J. Org. Chem.* 1958, **23**, 653.

1 (12) (a) Nakane, P. K.; Kawaoi, A. *J. Histochem. Cytochem.* 1974, **22**, 1084. (b) Nakamura, S.; Hayashi, S.; Koga, K. *Biochim. Biophys. Acta* 1976, **445**, 294.

1 (13) Sewickly, E.; Hauser, C. R.; Stanley, T. W.; Elbgi, W. *Anal. Chem.* 1961, **33**, 970.

1 (14) Glucose procedure 341, Sigma Chemical Co., St. Louis, MO.

1 (15) Royer, G. P. in *Methods in Enzymology, Immobilized Enzymes and Cells*; Colowick, S. P., Kaplan, N. O., Mosbach, K., Eds.; Academic Press: San Diego, CA, 1987; Vol. 135, p 141.

1 (16) Schuhmann, W.; Kitteiner, R. *Biosensor Bioelectronics*, in press. Presented at the First World Congress on Biosensor, Singapore, 1990.

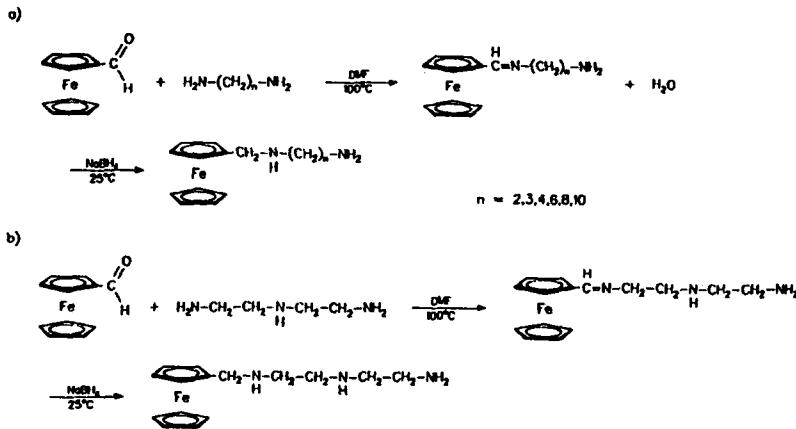
1 (17) Sewickly, E.; Schmeidler, R.; Engel, C. R. *Makromol. J.* 1967, **12**, 377.

Auth:
See MSS
Page 13

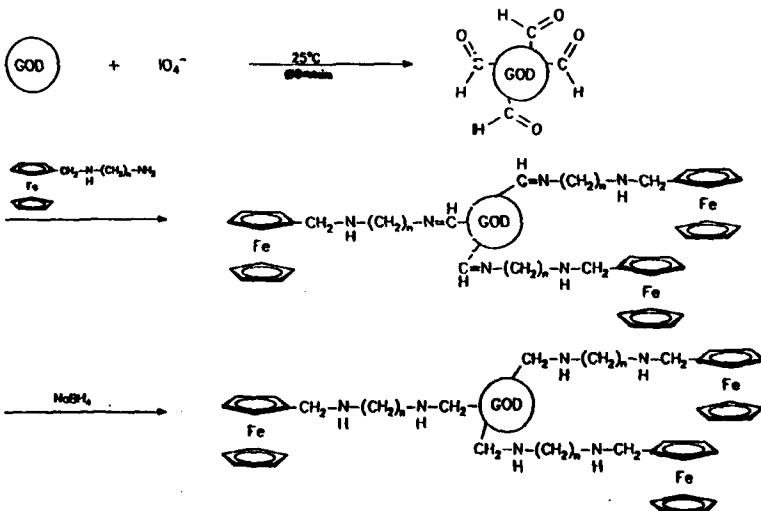
UNIT NO. 242
Gal. 5 JA1C1SM JA9015050 V113 1003

901207

FNN18
FNP51



CAP00 1 Figure 1. Synthesis of ferrocene amines with spacer chains for the separation of Tcdox and amine functions.



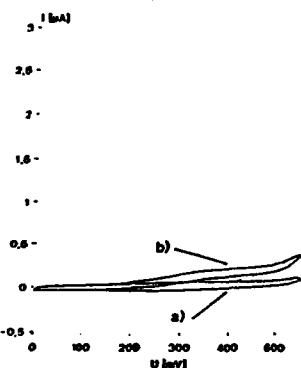
CAP00 1 Figure 2. Preparation of glucose oxidase modified by peripherally bound ferrocenes.

UNIT NO. 243
Gal. 6 JAICISM JA9015050 V113 1003

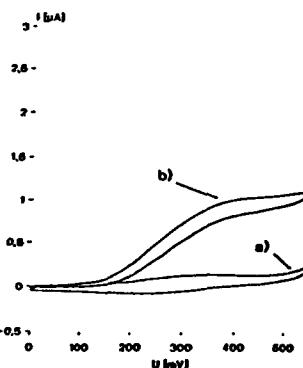
901207

FNN18
FNP51

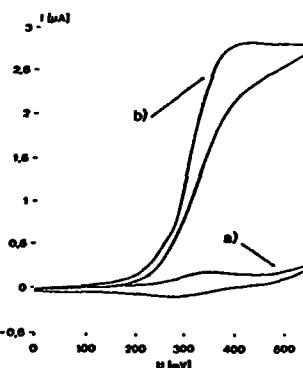
7 BOND CHAIN (1)



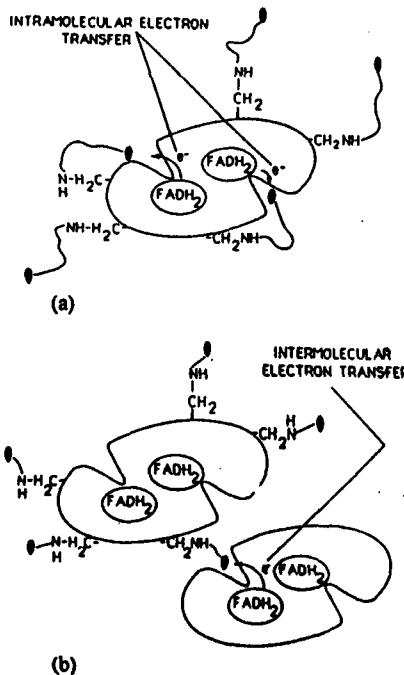
8 BOND CHAIN (2)



13 BOND CHAIN (4)



CAP00 1 **Figure 3.** Effect of the chain length connecting peripherally bound ferrocene to glucose oxidase on the electrocatalytic glucose oxidation current. Curves
CAP09 2 a represent oxidation currents in the absence of glucose; curves b represent currents at 40 mM glucose. All solutions contain 2 mg mL⁻¹ of one of the
12 modified enzymes, 0.1 M phosphate buffer (pH 7.2), and 200 units/mL⁻¹ catalase; 3-mm-diameter glassy carbon disks; all potentials vs SCE; scan
33 rate 10 mV s⁻¹.



CAP00 1 **Figure 4.** (a) Intramolecular and (b) intermolecular electron transfer via
CAP09 10 chain-attached mediators.

TTL20 IIDB40 Table I. Effect of the Spacer Chain Length on the Catalytic Current of Ferrocene-Modified Glucose Oxidase							
	no.	compound	bonds	i_{cat}^a nA	[Fc] _{rel} ^b	$i_{cat}/[Fc]_{rel}$	rel enzyme activ [O ₂] ^c
ROW50	1	Enz-CH ₂ -NH-(CH ₂) ₂ -NH-CH ₂ -Fc	7	200	1.50 ± 0.20	400 ± 160	0.27
ROW60	2	Enz-CH ₂ -NH-(CH ₂) ₃ -NH-CH ₂ -Fc	8	1010	1.00 ± 0.10	1010 ± 100	0.38
ROW70	3	Enz-CH ₂ -NH-(CH ₂) ₄ -NH-CH ₂ -Fc	11	1190	1.00 ± 0.10	1190 ± 120	0.45
ROW80	4	Enz-CH ₂ -NH-(CH ₂) ₅ -NH-CH ₂ -Fc	13	2800	1.00 ± 0.10	2800 ± 280	0.41
ROW90	5	Enz-CH ₂ -NH-(CH ₂) ₁₀ -NH-CH ₂ -Fc	15	2680	1.00 ± 0.10	2680 ± 270	0.49
ROW100	6	Enz-CH ₂ -NH-(CH ₂) ₂ -NH-CH ₂ -CH ₂ -Fc	5	460	0.75 ± 0.25	600 ± 200	0.33
ROW110	7	Enz-CH ₂ -NH-[(CH ₂) ₂ -NH] ₂ -CH ₂ -Fc	10	3200	1.00 ± 0.10	3200 ± 320	0.36

FNT120 *Catalytic glucose oxidation current on 3-mm-diameter glassy carbon electrodes at 0.35 V (SCE). ^aCoulometrically determined relative number of ferrocenes per enzyme. ^bHydrogen peroxide rate of formation, measured relative to the native glucose oxidase rate.

AID00 INITIAL TABLE WIDTH IS DOUBLE COLUMN

TTL20 **Table II.** Catalytic Current of Partially Deactivated Ferrocene-Modified Enzymes

IIDB40						
	no.	compound	bonds	i_{cat}^a nA	i'_{cat} (deactiv) ^b nA	i''_{cat} (deactiv + native cnz) ^c nA
ROW50	1	Enz-CH ₂ -NH-(CH ₂) ₂ -NH-CH ₂ -Fc	7	200	120	170
ROW60	4	Enz-CH ₂ -NH-(CH ₂) ₅ -NH-CH ₂ -Fc	13	2800	350	470

FNT70 *Catalytic current for modified enzyme from Table I. ^aCatalytic current for modified, then partially deactivated enzyme. ^bCatalytic current of (b) after add. ion of an equal amount (1 mg ml.⁻¹) of native glucose oxidase.

AID00 INITIAL TABLE WIDTH IS SINGLE COLUMN

The number of words in this manuscript is 2896.

The manuscript type is A.

Running Heads

Electron Transfer between Glucose Oxidase and Electrodes

Schuhmann et al.

Author Index Entries

Schuhmann, W.

Ohara, T. J.

Schmidt, H.-L.

Heller, A.

Text Page Size Estimate = 2.4 Pages

Graphic Page Size Estimate = 1.5 Pages

Total Page Size Estimate = 3.9 Pages